Journal of Medical Virology 54:135–139 (1998)

Prevalence of Antibodies to the Hawaii Strain of Human Calicivirus as Measured by a Recombinant Protein Based Immunoassay

W. David Cubitt,[1]* Kim Y. Green,[2] and Pierre Payment[3]

[1]*Department of Virology, Camelia Botnar Laboratories, Great Ormond Street Hospital for Children, London, United Kingdom*
[2]*National Institutes of Health, Bethesda, Maryland*
[3]*Institut Armand Frappier, Universite du Quebec, Laval, Canada*

The evaluation of an enzyme immunoassay using recombinant Hawaii virus–like particles (rHV-LPs) with a panel of sera which had been screened previously for antibodies to Norwalk virus (NV) and Mexico virus (MxV) is described. The assay was also applied to study the epidemiology of Hawaii virus. Adult volunteers challenged with the prototype (genogroup II, human calicivirus) HV developed significant IgG responses (16–32 fold rises) following challenge whereas adults challenged or naturally infected with NV (genogroup I) did not. Lesser antibody responses (4–8 fold rises) were demonstrated in volunteers challenged with Snow Mountain agent (SMA) and patients infected by SRSV 'UK3' and 'UK4' strains, indicating a degree of antigenic relatedness among viruses within genogroup II. Comparison of the seroprevalence of Ig G antibodies to rHV, rMxV and rNV in 338 children in London showed that infections with genogroup II viruses are prevalent and occur earlier in life than NV. Many young children had antibodies to MxV but not HV indicating that genogroup II viruses have both conserved and antigenically distinct epitopes. A serological study on 566 Canadians aged between 9 and 79 years showed that the prevalence of antibodies to rHV rose with age from 65–100% and from 53–100% for NV. Measurement of antibody response in a heart transplant patient infected with an MxV-like virus showed significant responses to both rMxV and rHV. Continuous monitoring of the patient over two years showed that antibody levels declined rapidly to prechallenge levels after a year. ***J. Med. Virol. 54:135–139, 1998.***

KEY WORDS: Hawaii virus; Mexico virus; Norwalk virus; calicivirus; epidemiology; transplant; Canada

INTRODUCTION

Hawaii virus (HV) was first described following an investigation into the cause of an outbreak of gastroenteritis involving children and adults, which occurred in Honolulu in March 1971. Subsequently, cross challenge studies in adults suggested that HV was antigenically distinct from Norwalk virus (NV) and demonstrated that the presence of preexisting antibodies failed to prevent volunteers becoming ill [Wyatt et al., 1974]. Further evidence that HV, NV and Snow Mountain agent (SMA) were antigenically distinct was provided by studies using immune electronmicroscopy [Dolin et al., 1982] and specific enzyme immunoassays (EIA) [Madore et al.,1990; Treanor et al., 1993]. Numerous serological studies have been conducted using NV derived from adult volunteers [reviewed by Kapikian, 1994] and more recently using virus-like particles (VLPs) expressed in baculovirus [Jiang et al., 1992; Parker et al., 1993, 1994; Gray et al., 1993; Numata et al., 1994]. However, due to limited stocks of antigen there is an absence of similar data on the seroprevalence of HV.

In 1994, HV was genetically characterised and shown to be a human calicivirus (HuCV) with a sequence that places it within 'genogroup II' together with Toronto virus (TV), Mexico virus (MxV) [Berke et al., 1997] and several SRSVs typed by solid phase immune electronmicroscopy as 'UK3' or UK4' [Green et al., 1995]. It was also shown to be distinct from the genogroup I viruses (NV, Southampton virus and Desert Shield virus) that share only 48% amino acid identity with HV in the composition of their capsid protein [Lew et al., 1994]. Since then HV VLPs have been expressed in baculovirus [Green et al., 1997b] which led

*Correspondence to: W. David Cubitt, Dept. of Virology, Camelia Botnar Laboratories, Hospital for Children, Great Ormond St., London WC1 N 3JH, UK. E-mail:d.cubitt@ich.ucl.ac.uk

Accepted 6 October 1997

to the development of EIAs for the investigation of HV infections.

The present study describes the development and evaluation of an EIA to detect HV specific IgG antibodies. The EIA was used to ascertain whether or not SRSV UK3 and UK4 strains were antigenically related to HV. The assay was then applied to determine if HV was cause of a number of community outbreaks of gastroenteritis in adults and to perform seroprevalence studies on populations from England and Canada.

MATERIALS AND METHODS

Sera

Details of the origin of the sera analysed in the current study which had been tested previously for the presence of Ig G antibodies to NV and MxV have been published [Parker et al., 1993, 1994, 1995]. Pre- and post-infection sera were obtained from two adult volunteers who had been challenged with HV and two volunteers who had been challenged with SMA. These sera had previously been tested in the rMxV and rNV antibody EIAs [Jiang et al., 1995]. Reference sera were obtained from adult volunteers challenged with NV and from community outbreaks caused by NV-like viruses. Paired sera were available from patients known to have been infected with caliciviruses originally typed as SRSV UK3 and UK4 strains [Lewis, 1991] many of whom were found to be excreting virus particles. Paired sera were available from patients involved in community outbreaks of gastroenteritis which occurred in the UK, USA and Canada. Sera from a cohort of 338 children aged 1 day to 16 years referred for virological tests to Great Ormond Street Hospital for Children, London. Details of the origin of all the sera described above which had previously been tested for the presence of Ig G antibodies to NV and MxV have been published [Parker et al., 1993, 1994, 1995].

In addition tests were carried out on a series of 19 sera collected over a period of 2 years from a heart transplant patient who was infected with a genotype II HuCV, (12C/92/UK) that was found to be related genetically and antigenically to MxV [Cubitt et al., 1994; Jiang et al., 1995].

Samples from 566 individuals aged 9–79 years who were enrolled in a survey in 1988 to monitor the efficacy of water filtration, in Quebec, Canada were tested. These sera had previously been screened for the presence of antibodies to NV [Payment et al., 1994].

Antigen: Hawaii Virus-Like Particles (rHV.VLPs)

Recombinant baculovirus expressing rHV.VLPs [Green et al., 1997a] was passaged in SF 21 cells. Once a cytopathic effect was observed, cells were harvested and resuspended in phosphate buffered saline. The cell debris was removed by low speed centrifugation and the supernate which was shown by electronmicroscopy to contain large numbers of VLPs used as the source of antigen. A series of titrations of antigen versus antibody were undertaken to determine the optimal concentration for use of the antigen.

Enzyme Immunoassay for HV IgG

The method employed was as described previously for testing for IgG specific antibodies to NV [Parker et al., 1993] and Mexico virus (MxV) [Parker et al., 1995] using recombinant VLPs. Sera from volunteers, individuals involved in community outbreaks and from the transplant patient were tested in a series of doubling dilutions from 1:100 through 1:51,200. Endpoints were taken as the highest dilution at which an A_{450nm} T-N value of >0.2 and a T/N value of >2.0 was obtained. Sera from the 338 children were tested at a single dilution of 1:100 and the titres estimated by comparison with a back titration of a sample shown to have a titre of 1:51,200 and a calibration curve obtained by comparing the OD at a dilution of 1:100 with the endpoints obtained by titration of 52 sera. A >8-fold antibody response to HV was considered to be significant.

RESULTS

The results of testing four samples in parallel in series of doubling dilutions from 1:100 through 1:12,800 using wells coated with a series of log dilutions of the HV antigen suspension ranging from 10^{-1} through 10^{-4} showed the optimal concentration for coating to be 10^{-3}.

The two adult volunteers challenged with HV showed significant IgG antibody responses to rHV, (Volunteer 86–33, 800–204,800 [256 fold rise]; Volunteer 86–38, 1,600–51,200 [32 fold rise]). Antibody responses to rHV were also demonstrated in the two volunteers challenged with SMA, (Volunteer 86–25, 1,600–12,800 [8 fold rise]; Volunteer 86–28, 6,400–25,600 [4 fold rise]).

Tests on pre- and post-challenge sera from thirteen adult volunteers who developed >16 fold antibody responses to rNV following infection with NV [Parker et al., 1993] showed that they all had preexisting antibodies to rHV (IgG titres, 400–>25,600). The levels of HV specific IgG remained unchanged in ten individuals but the other three showed slight (2-fold rises) which were not considered to be significant.

Significant responses (>8 fold) to NV but not to HV or MxV were found in patients involved in outbreaks caused by consumption of contaminated oysters, NV/Melbourne/78/Aus, SRSV/London/88/UK [Parker et al., 1993] and a community outbreak caused by a virus identified as SRSV/UK2 [Lewis, 1991].

The results of tests on acute and convalescent phase sera from patients involved in three outbreaks in which patients were excreting viruses (typed as SRSV/UK3) (Table I) showed that 3/6 patients developed 4 fold increases in IgG levels to HV, two of whom also showed rising (2 fold Ig G antibody rises) to MxV.

A patient involved in the SRSV/UK4/Leeds/91 outbreak (Table II) showed significant IgG responses to rHV (8 fold rise) and to rMxV (16 fold rise) and a slight elevation in levels of rNV IgG (2 fold rise). A similar response was demonstrated in one patient involved in the San Anita outbreak.

Paired sera from patients involved in a further 12 outbreaks caused by untyped strains which occurred in

TABLE I. IgG Responses to Hawaii, Mexico and Norwalk Viruses in Patients Involved in Outbreaks Caused by SRSV UK3 Strains*

Outbreak	Serum	rHV	rMxV	rNV
UK3/Barnet/86/UK	1164 A	51200	6400	32000
	1714 C	25600	6400	32000
	1163 A	25600	12800	4000
	1717 C	51200	12800	4000
UK3/Blake/UK	9887 A	**1600**	1600	1000
	11076 C	**6400**	3200	2000
UK3/PretPath/91/SA	WOK A	3200	800	16000
	WOK C	6400	800	8000
	JU A	**3200**	800	8000
	JU C	**12800**	800	8000
	CS A	**1600**	100	<1000
	CS C	**6400**	200	<1000

*A = Acute; C = convalescent.

TABLE II. SRSV Outbreaks in Which Patients Developed Significant (⩾8 fold) IgG Responses to rHV*

Outbreak	Serum	rHV	rMxV	rNV
UK4/Leeds/91/UK	1A	**800**	**3200**	8000
	1C	**6400**	**51200**	16000
San Anita/80/US	1A	**100**	**400**	4000
	1C	**800**	**6400**	4000
	2A	100	**1600**	NA
	2C	200	**12800**	NA
Wharfedale/93/UK	1A	**1600**	NA	NA
	1C	**12800**	NA	NA
Lenoir/87/Can	1A	**3200**	400	<1000
	1C	**204800**	400	2000
	2A	**800**	800	<1000
	2C	**6400**	1600	<1000

*A = Acute; C = Convalescent; NA = Not available.

TABLE III. Seroprevalence (%) of IgG Antibodies to Norwalk and Hawaii Viruses by Age Group in 566 Canadians

Age group	Number in group	NV+	HV+	NV & HV+	NV & HV−
9–19	75	53	65	37	19
20–29	37	59	72	48	16
30–39	165	82	82	64	5
40–49	208	87	86	75	2
50–59	70	81	83	71	6
>60	11	100	100	100	0

the UK and North America between 1980–1993 showed that the majority (68%) of adults had preexisting antibodies to rHV. Significant (8–64 fold) IgG antibody responses to rHV, similar to those observed in volunteers challenged with HV, were detected in patients involved in only two of these outbreaks (Table II). The patient involved in the 'Wharfedale' outbreak caused by a virus that had 93% amino identity with HV over a 1,500 bp region encoding the capsid protein (Green J and Lewis D, personal communication) showed an 8 fold rise to rHV; insufficient sample was available to run tests for antibodies to MxV or NV.

Tests on sera obtained from a heart transplant recipient shown to be excreting HuCV/12C/92/UK (an MxV-like virus) demonstrated that prior to infection (Days -32 & -22) low levels of IgG (400–800) to rHV, rMxV and rNV were present in his sera. During the 3-week period following infection he developed a strong serological response (800–25,600) to rMxV and a lesser response (1,600–6,400) to rHV. However, there was no significant response to rNV (1,600–3,200). On day 24 post infection, he was diagnosed as having a primary CMV infection at which point titres to all three viruses dropped to preinfection levels indicative of immune suppression. During the following two weeks following treatment with gancyclovir his IgG levels to rMxV and rHV began to rise again; peaking at a titre of 25,600 to HV on Day 51 and at 51,200 on Day 72 for MxV. Over the following eight months the titres of antibody to all three viruses declined, returning after a year to preinfection levels and persisting without change over the following two years. Throughout the period of monitoring he showed no significant response to rNV; IgG titres remaining within the range 400–1,600. Peak titres of 1,600 of NV IgG occurred on days 20 and 72 corresponding to the peak IgG levels to MxV.

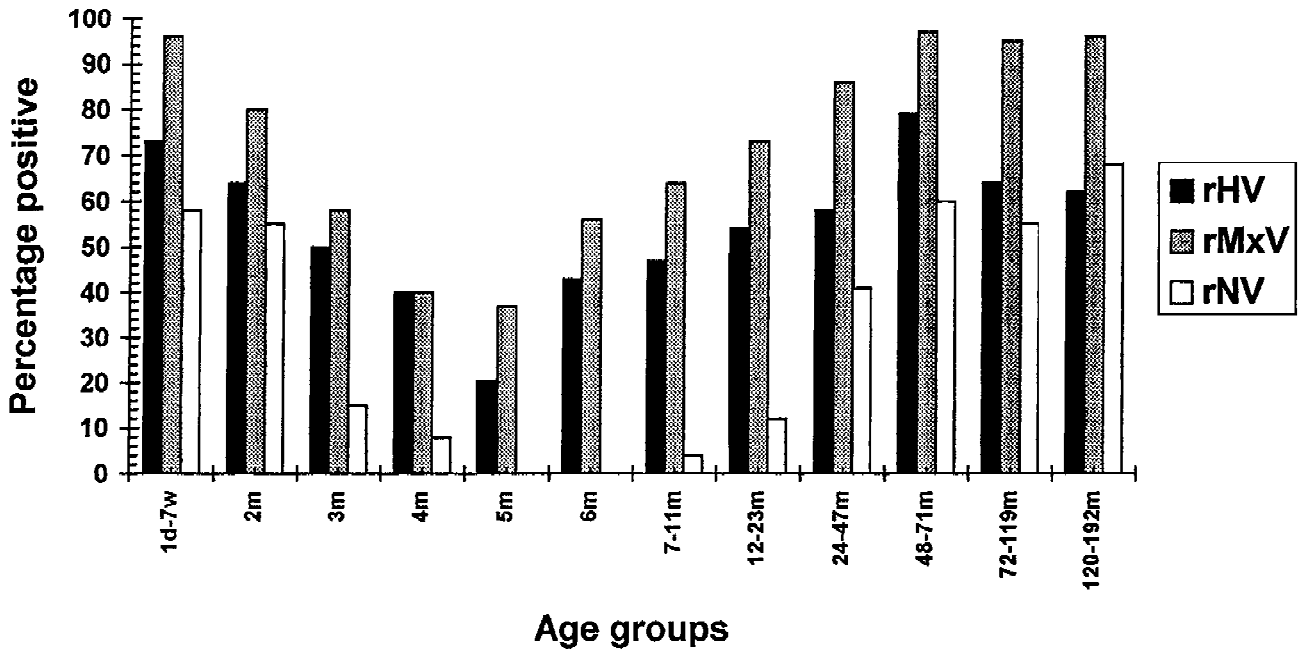

Fig. 1. Prevalence of IgG antibodies to Hawaii, Mexico and Norwalk virus in 338 children in London, UK.

A calibration curve enabling the titre to be predicted by testing sera at a single dilution (1:100) was obtained by plotting the optical densities at A 450 nm of 52 sera at 1:100 against their endpoints, obtained by serial dilution until an OD of 0.2 was achieved. A log–linear relationship was found between the OD and endpoint, correlation coefficient 0.997, R-squared 0.995.

The seroprevalence and relative age of acquisition of IgG antibodies to HV, MxV and NV in 338 children from London are shown in Figure 1. The prevalence of antibody to HV was 73% (mean titre = 2,713) in neonates aged <2 months decreasing to 20% (mean titre = 60) in children aged 5 months. Thereafter children gradually acquired infection the prevalence rising to 79% (mean titre = 1,357) in the age group 4–5 years, paralleling the pattern observed for the other genotype II virus, MxV. Only one child aged 1–3 year had evidence of exposure to HV but not to MxV. However, many children possessed IgG antibodies to MxV but not HV or NV indicating that MxV infections are more common and that each virus is antigenically distinct.

Comparison of the seroprevalence of antibodies to HV and NV in 566 Canadians aged between 9 and 79 years are shown in Table III. The prevalence rate of HV rose from 65% in the youngest group to 100% in the elderly. There was a similar rising trend in the number of individuals who had evidence of exposure to both NV and HV. Antibody titres in seropositive individuals ranged from 100–12,800 (median = 1,600).

DISCUSSION

The antibody responses in two adult volunteers challenged with the prototype HV showed that they both developed a significant antibody response (32 and 256 fold rises) to rHV. Previous studies [Jiang et al., 1995] showed that both volunteers also produced significant but lesser antibody responses to rMxV (8 and 16 fold rises) but not to rNV.

Antibody responses to rHV (8 and 4 fold rises) were detected in the two adults challenged with SMA but greater responses (64 and 16 fold rises) were demonstrated to rMxV [Jiang et al., 1995]. These results together with phylogenetic data suggest that SMA is more closely related to MxV than HV. The antibody responses in the heart transplant patient infected with an Mx-like virus also demonstrate a significant degree of cross reaction between HV and MxV. These findings agree with the previous observations [Madore et al., 1990] who used antigen derived from volunteers, that there is a degree of antigenic relationship between genogroup II caliciviruses, i.e. HV and SMA. However, the seroepidemiological surveillance data show that following primary infection many children possessed antibodies to MxV but not HV. In addition two adults who showed significant seroresponses to HV in the 'Lenoir' outbreak had poor responses to rMxV. The results of tests on paired sera from patients involved in three outbreaks associated with viruses which were typed as SRSV 'UK3's by SPIEM either showed low (4 fold) or unchanging IgG responses to rHV and rMxV. This suggests that SRSV 'UK3' strains are more distantly related to HV than the SRSV/UK4/Leeds virus, although they fall within the genogroup II clade, [Ando et al., 1995]. Alignment of the complete capsid protein sequences of HV and MxV shows 76% amino acid conservation and therefore it is not surprising that there is a degree of antigenic relatedness. However, the presence of individuals with antibody to MxV but not HV indi-

cates that there must also be distinct antigenic epitopes which are most likely to exist in the central hypervariable region of ORF2 [Green et al., 1995, 1997a].

The seroresponses in adult volunteers and patients infected with Norwalk virus or SRSV 'UK2' strains showed a low level of antigenic relatedness between HV and genogroup I viruses similar to the results obtained when the sera were analyzed in the rMxV EIA [Parker et al., 1995] and observations in cross challenge experiments, [Madore et al., 1990; Treanor et al., 1993]. Further evidence for a lack of antigenic relatedness is evident from the Canadian seroprevalence study in which only 37% of individuals aged 9–19 years possessed antibodies to both HV and NV.

The seroprevalence data from both England and Canada show that viruses antigenically related to HV, MxV and NV are circulating commonly in the community and that infections with genogroup II viruses occur earlier in life. These observations are consistent with data based on capsid sequences which indicate that many strains are cocirculating in the general population in both infants and adults [Green et al., 1997a].

In conclusion the results confirm the previous observations that there are significant antigenic differences between the genogroup I and II viruses. However, the relatively high degree of antigenic relatedness between MxV, SMA and HV often makes it difficult to establish which strain is the cause of an outbreak in adults based on the results of a single EIA. It is also evident that paired sera from many individuals involved in an outbreak need to be screened in order to make a firm serological diagnosis. A previous study [Green et al., 1997b] clearly demonstrated that in the majority of outbreaks of gastroenteritis that they analysed only a small percentage of infected individuals mounted a significant response to rHV, suggestive of infection with other strains of calicivirus.

A recent study from the UK demonstrates that several strains of genogroup I and II viruses have been circulating in the community over the past eight years [Green et al., 1997a].

Further studies are in progress to ascertain whether or not IgM and IgA responses would provide a more definitive diagnosis as has been found to be the case in NV infections [Treanor et al., 1993; Monroe et al., 1993; Parker et al., 1994].

REFERENCES

Ando T, Monroe SS, Gentsch JR, Jin Q, Lewis DC, Glass RI (1995): Detection and differentiation of antigenically distinct small round structured viruses (Norwalk-like viruses) by reverse transcriptase polymerase chain reaction and southern hybridization. Journal of Clinical Microbiology 33:64–71.

Berke T, Golding B, Jiang X, Cubitt WD, Wolfaardt M, Smith AW, Matson DO (1997): A phylogenetic analysis of the caliciviruses. Journal of Medical Virology 52:419–424.

Cubitt WD, Jiang XJ, Wang J, Estes MK (1994): Sequence similarity of human caliciviruses and small round structured viruses. Journal of Medical Virology 43:252–258.

Dolin R, Levy AG, Wyatt PG, Thornhill TS, Gardner JD (1975): Viral gastroenteritis induced by the Hawaii agent. Jejunal histopathology and serologic responses. American Journal of Medicine 59: 761–768.

Dolin R, Reichmann RC, Roessner KD, Tralka TS, Schooley RT, Gary W, Morens D (1982): Detection by immune electronmicroscopy of the Snow Mountain agent of acute viral gastroenteritis. Journal of Virological Methods 22:207–214.

Gray JJ, Jiang X, Morgan-Capner P, Estes MK (1993): Prevalence of antibodies to Norwalk virus in England. Detection by enzyme linked immunosorbent assay using baculovirus expressed Norwalk virus capsid antigen. Journal of Clinical Microbiology 31: 2185–2191.

Green KY, Kapikian AZ, Valdesuso J, Sosnovtsev S, Treanor JJ, Lew JF (1997a): Expression and self-assembly of recombinant capsid protein from the antigenically distinct Hawaii human calicivirus. Journal of Clinical Microbiology 35:1909–1914.

Green SM, Lambden PR, Caul EO, Ashley CR, Clarke IN (1995): Capsid diversity in small-round structured viruses: molecular charcterisation of antigenically distinct human enteric calicivirus. Virus Research 37:271–283.

Green SM, Lambden PR, Caul EO, Clarke IN (1997b): Capsid sequence diversity in small round structured viruses from recent UK outbreaks of gastroenteritis. Journal of Medical Virology 52:14–19.

Jiang X, Cubitt WD, Hu J, Xiaming D, Treanor J, Matson DO, Pickering LK (1995): Development of an ELISA to detect MX virus, a human calicivirus in the Snow Mountain agent genogroup. Journal of General Virology 76:2739–2747.

Jiang X, Matson DO, Ruiz-Palacios GM, Hu J, Treanor J, Pickering LK (1995): Expression, self assembly and antigenicity of a Snow Mountain agent-like calicivirus capsid protein. Journal of Clinical Microbiology 33:1452–1455.

Jiang X, Wang M, Graham DY, Estes MK (1992): Expression, self assembly and antigenicity of the Norwalk virus capsid protein. Journal of Virology 66:6527–6532.

Kapikian AZ (1994): Norwalk and Norwalk-like viruses. In 'Viral infections of the gastrointestinal tract' 2nd Edition. pp 471–518. (ed) Kapikian AZ. Marcel Dekker.

Lew JF, Kapikian AZ, Valdesuso J, Green KY (1994): Molecular characterization of Hawaii agent and other Norwalk-like viruses: evidence for genetic polymorphism among human caliciviruses. Journal of Infectious Diseases 170:535–592.

Lewis DC (1991): Norwalk virus and other small round structured viruses in the UK. Journal of Infection 23:220–222.

Madore HP, Treanor JJ, Bija R, Dolin R (1990): Antigenic relatedness among the Norwalk-like agents by serum antibody rises. Journal of Medical Virology 32:96–101.

Monroe SS, Stine SE, Jiang X, Estes MK (1993): Detection of antibody to recombinant Norwalk virus antigen in specimens from outbreaks of gastroenteritis. Journal of Clinical Microbiology 31: 2866–2872.

Numata K, Nakata S, Jiang X, Estes MK, Chiba S (1994): Epidemiological study of Norwalk virus infections in Japan and southeast Asia by enzyme linked immunosorbent assays with Norwalk virus capsid protein produced by baculovirus expression system. Journal of Clinical Microbiology 32:121–126.

Parker SP, Cubitt WD (1994a): Measurement of IgA responses following infection with Norwalk virus and other human caliciviruses using a recombinant Norwalk virus protein EIA. Epidemiology and Infection 113:153–159.

Parker SP, Cubitt WD, Jiang X, Estes MK (1994b): Seroprevalence studies using a recombinant Norwalk virus protein enzyme immunoassay. Journal of Medical Virology 42:146–150.

Parker SP, Cubitt WD, Jiang X (1995): An enzyme immunoassay using baculovirus expressed human calicivirus (Mexico) for the measurement of IgG responses and determining its seroprevalence in London UK. Journal of Medical Virology 46:194–200.

Treanor JJ, Jiang X, Madore HP, Estes MK (1993): Subclass specific serum antibody responses to recombinant Norwalk virus capsid antigen (rNV) in adults infected with Norwalk, Snow Mountain or Hawaii virus. Journal of Clinical Microbiology 31:1630–1634.

Treanor JJ, Madore HP, Dolin R (1988): Development of an enzyme immunoassay for the Hawaii agent of viral gastroenteritis. Journal of Virological Methods 22:207–214.

Wyatt RG, Dolin R, Blacklow NR, Du Pont H, Buscho RF, Thornhill TS, Kapikian AZ, Chanock RM (1974): Comparison of three agents of acute infectious non-bacterial gastroenteritis by cross challenge in volunteers. Journal of Infectious Diseases 129:709–714.